

Table 5: Anti-PA and the neutralizing antibody titer generated by vaccination of rats with PA mutants

	Animals	Anti-PA Titer	Neutralizing Titer	TTM
PBS	6	< 10	< 10	74.2 ± 1.5
WT	5	43,300	2,490	Survived
5 ΔD2L2	6	47,500	3,350	Survived
K397D + D425K	6	65,500	2,260	Survived
F427A	6	132,000	6,090	Survived

Example 10: Antibodies to PA

- 10 Antibodies to a PA protein may be used as therapeutics and/or diagnostics. Antibodies may be produced using standard methods by immunologically challenging a B-cell-containing biological system, *e.g.*, an animal such as a mouse or rabbit, with a PA protein or a fragment thereof to stimulate production of an

may be obtained, for example, by affinity chromatography using recombinantly-produced protein or conserved motif peptides and standard techniques.

By “specifically binds” is meant an antibody that recognizes and binds to, for example, wild-type PA or a PA mutant but does not substantially recognize and bind to other non-PA molecules in a sample, *e.g.*, a biological sample, that naturally includes protein. A desirable antibody specifically binds any of the PA mutants # 1-18 in Table 1. Other desirable antibodies bind wild-type PA with at least 2, 5, 10, or 20 fold greater affinity than they bind one or more of the PA mutants in Table 1.

Sequence identity is typically measured using sequence analysis software with the default parameters specified therein (*e.g.*, Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705). This software program matches similar sequences by assigning degrees of homology to various substitutions, deletions, and other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine, valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine.

Other features and advantages of the invention will be apparent from the following detailed description.

Brief Description of the Drawings

Fig. 1 is a schematic illustration of the intoxication pathway for ATx toxin. The PA component of ATx binds to a receptor on the surface of mammalian cells and delivers the enzymic A moieties of the toxin, edema factor (EF) and lethal factor (LF), to the cytosol, as described above.

or an adjuvant. The vaccine can be administered orally, intramuscularly, intravenously, subcutaneously, by inhalation, or by any other route sufficient to provide a dose adequate to prevent or treat a bacterial infection. In another desirable embodiment, a vaccine that includes a mutant anthrax protective antigen is administered for the prevention or treatment of anthrax infection. In one embodiment, specifically excluded from this aspect is a method that involves administration of a vaccine having Δ D2L2 PA as its sole mutant B moiety and that does not involve administration of another vaccine having another mutant B moiety.

In a fourth aspect, the invention provides a mutant B moiety of a pore-forming binary A-B toxin. The mutant B moiety has a mutation that results in inhibition of its pore-forming ability. The mutant B moiety also inhibits the pore-forming ability of a naturally-occurring B moiety of the corresponding toxin *in vitro* and/or *in vivo*. In one desirable embodiment, this mutation results in inhibition of the pore-forming ability of the protein *in vivo*. In another desirable embodiment, the mutant B moiety lacks pore-forming ability *in vitro* and/or *in vivo*. In yet another desirable embodiment, the B moiety is anthrax protective antigen (PA). The mutant B moiety may bind the A moiety of the corresponding toxin. For example, a PA mutant may bind the lethal factor or edema factor A moieties. The mutant B moiety may compete with a naturally-occurring B moiety for binding to a receptor on the surface of a mammalian cell. The mutant B moiety may also bind a naturally-occurring B moiety of the corresponding toxin. Such a mutant may oligomerize with a naturally-occurring B moiety to form a complex that has reduced ability to form a pore. In one desirable embodiment, the complex lacks the ability to form a pore and to translocate an A moiety (*e.g.*, EF or LF) across the membrane into the host cell cytoplasm.

cleavage of a 20 kDa N-terminal segment from the PA polypeptide. PA63 forms a heptameric prepore and binds the two alternative A moieties, edema factor (EF) and lethal factor (LF). The entire complex is trafficked to the endosome, where PA63 inserts into the membrane, forms a transmembrane pore, and translocates EF and LF into the host cell cytoplasm.

“Transmembrane pore” means a transmembrane aqueous channel. For example, the transmembrane pore can be a β -barrel channel formed by alternating hydrophilic and hydrophobic residues of PA63 such that the hydrophobic residues form an exterior membrane-contiguous surface of the barrel, and the hydrophilic residues face an aqueous lumen of a pore that spans across the host cell membrane.

“Hydrophilic face of a transmembrane pore” means the amino acids of PA that face the aqueous lumen of a pore that spans across the host cell membrane.

“An amino acid that forms the transmembrane pore” means an amino acid of PA that is located in a β -barrel channel of a transmembrane pore.

“D2L2 loop” means the amphipathic loop which connects strands 2 β 2 and 2 β 3 of PA polypeptide and PA63 polypeptide as described herein.

“Inhibits the pore-forming ability” means reduces the amount of pores formed in membranes or reduces the rate or amount of an A moiety (*e.g.*, EF or LF) that is translocated into the host cell cytoplasm. This decrease in pore formation or toxin translocation is positively correlated with, and could be predicted by, a decrease in activity in the cell surface translocation, LFnDTA toxicity, or rubidium release assays described herein. This decreased activity can be correlated with a decrease in the amount of a radiolabeled ligand that is translocated into cells in the cell surface translocation assay, a decrease in the